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## Short Communication:

### The influence of pH on losses of analyte estradiol in sample pre-filtration

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#### Abstract

Cellulose acetate 0.45 µm syringe filters could be used to filter environmental samples containing steroidal hormones, such as estradiol, prior to analysis. Such pre-treatment may result in significant loss of analyte due to either adsorption of analyte to the filter material or association with retained colloidal and particulate materials. To determine the quantity of such losses, several experiments were performed by filtering water spiked with estradiol at different concentrations (50, 100, 500 ng/L) and pH (3-12) through cellulose acetate syringe filters. Further, the influence of added organic matter was studied. At all concentrations the experiments indicated that below pH 11, up to 50% of estradiol in the feed was adsorbed on the filter. This occurred regardless of the presence of bulk organic matter in solution, and was related to dissociation of estradiol with pH and subsequent changes in filter interaction. As a consequence pre-treatment of solutions containing estradiol should be adjusted to greater than pH 11 prior to filtration with 0.45 µm cellulose acetate filters.

**Keywords:** Sample pre-treatment, cellulose acetate syringe filters, estradiol

#### Introduction

Micropollutants, such as pesticides, hormones and pharmaceuticals, are ubiquitous in the aquatic environment and can be considered a cause of concern for both human and ecosystem health (Schwarzenbach *et al.*, 2006). Estradiol is a natural hormone which is excreted by humans and many animals, and as a result it can be considered ubiquitous in the aquatic environment (D'Ascenzo *et al.*, 2003; Sumpter and Jobling, 1995; Ternes *et al.*, 1999). Estradiol is of interest to many researchers as it is a potent hormone, and laboratory experiments have demonstrated that low concentrations of estradiol (nanograms per litre) can adversely affect aquatic organisms (Thorpe *et al.*, 2007; Thorpe *et al.*, 2000). Using a variety of analytical techniques, including gas and liquid chromatography, estradiol can be detected at sub nanogram per litre concentrations (Jeannot *et al.*, 2002; Rodriguez-Mozaz *et al.*, 2004; Williams *et al.*, 2003). Previous studies have used 0.45 µm cellulose acetate filters to pre-treat surface and groundwater samples containing steroidal hormones, such as estradiol, in acidic and neutral solutions prior to chromatography (Drewes *et al.*, 2005; Mansell and Drewes, 2004). Traditionally, 0.45 µm filters were used to distinguish between dissolved and particulate material (Clesceri *et al.*, 1998; Frimmel, 1998). Within the literature cellulose acetate filters have been used to pre-treat a large variety of sample types in the analysis of different compounds prior to chromatography (Furusawa, 1999), fluorescence spectroscopy (Jung

and Batley, 2004) and capillary electrophoresis (Karlsson *et al.*, 1999) as it could remove particulate matter which may interfere with analysis. As syringe filters were used for sample pre-treatment it was important to determine if estradiol adsorbed to such cellulose acetate filters, as this would reduce compound recovery. The influence of pH was also studied, as this could affect the interaction of estradiol with the filter due to a change in estradiol charge. Estradiol has a dissociation constant (pK<sub>a</sub>) of 10.23 (Kwon *et al.*, 2006), so it is negatively charged above pH 10. In addition different bulk organic compounds, such as natural organic matter (NOM) surrogates and polysaccharides, were selected to determine whether their presence would have an impact on estradiol adsorption, and hence analyte recovery. Therefore, the purpose of this study was to determine if cellulose acetate syringe filters were a suitable pre-treatment option for the analysis of estrogens.

#### Experimental

##### Chemicals and Standards

All chemicals were of analytical grade. The background electrolyte was 1 mM NaHCO<sub>3</sub> 20 mM NaCl and pH was adjusted from 3 to 12 using 1 M NaOH and HCl (Sigma Aldrich, Gillingham, UK). All experiments were conducted in de-ionized water. Radiolabelled [2,4,6,7-<sup>3</sup>H]estradiol (3.15 TBq/mmol, 37 MBq/mL) was purchased from GE Healthcare (Bucks, UK). 100 µg/L estradiol stock solutions were prepared in methanol. The concentration of estradiol used in the experiments was 50, 100 and 500 ng/L (results from 100 ng/L are shown in this paper only). Aldrich humic acid (HA) and alginic acid (sodium salt) were purchased from Sigma Aldrich. Suwannee River reference IHSS natural organic matter (NOM) was purchased from the International Humic Substance Society (St Paul's, USA), while Australian NOM was concentrated using microfiltration and reverse osmosis from Brisbane Water National Park, Australia (Schäfer, 2001). The concentration of carbon in all experiments was 12.5 mg of carbon per litre (mgC/L), with the exception of Aldrich HA, where experiments were conducted at both 5 and 12.5 mgC/L.

##### Sample Preparation and Filtration

Bulk organic matter and estradiol were added to 100 mL flask with background electrolyte and the pH was adjusted. The solution was shaken for 48 hours using a Sartorius Certomat BS-1 incubator shaker (Göttingen, Germany) at 200 rpm and a temperature of 25 °C to ensure equilibrium between estradiol and organic matter was reached. Estradiol degradation over 72 hours was explored by Neale *et al.* (2008) and found to be negligible, therefore it was unlikely estradiol degraded significantly during the experiment. 10 mL of solution was removed and filtered through a 0.45 µm cellulose acetate filter using a 10 mL syringe. The cellulose acetate syringe filters (0.45 µm LCW 916, 26 mm diameter) were purchased from Hach Lange GmbH (Düsseldorf, Germany). To determine estradiol adsorption, any material retained on the filter was desorbed using 10 mL of methanol using a dedicated syringe.

##### Analysis

1 mL of each of the feed, permeate and methanol used to desorb the filter were transferred to a 20 mL glass scintillation vials containing 7 mL of Ultima Gold LLT (Perkin Elmer, Waltham, USA). The methanol was not evaporated prior to analysis. The samples were analysed using a Beckman LS 6500 liquid scintillation counter (Fullerton, USA). The activity of the samples in disintegrations per minute (dpm) was converted to mass of estradiol (nanograms) in feed and permeate and mass desorbed from the filter. In addition, the mass absorbed to the filter was also calculated using mass balance. Estradiol adsorption to the plastic syringe was measured, but was shown to be negligible. Using error propagation (Equation 1), the random error (%E<sub>total</sub>) associated with the experiments was 5.31%. %E represents the variability associated with the analytical equipment, including the syringe used for methanol desorption, micropipettes and electronic balances, which were estimated to be 5%, 0.6% and 1% respectively.

$$\%E_{total} = \sqrt{(\%E_1)^2 + (\%E_2)^2 + (\%E_3)^2} \quad (1)$$

## Results and Discussion

### Mass of Estradiol in Permeate

The mass of estradiol in the permeate was graphed as function of pH and bulk organic matter type for 100 ng/L estradiol concentration (Figure 1). The mass in the permeate remained constant from pH 3 to 10 with approximately 50% of the total mass of estradiol recovered in the permeate. However, at pH 11 and 12 between 80-100% of the total mass of estradiol was present in the permeate. For all estradiol concentrations (50, 100 and 500 ng/L) the same trend was observed indicating concentration had no influence on results, therefore results for 100 ng/L were shown only.

Results from filtration in the presence of organic matter were similar; the mass of estradiol in the permeate from pH 3 to 10 was around 50%. In addition, when the pH was adjusted to 11 and 12 the mass of estradiol in the permeate was between 80-100%. This was observed for all bulk organics and at all concentrations. The molecular weights of all bulk organics studied were considerably smaller than the pore size of the cellulose acetate filter, therefore none of the bulk organic matter could be physically retained by size exclusion. However, adsorption of bulk organic matter to the filter may be possible as indicated by Fan *et al.* (2001).

Estradiol retention by such filters was due to adsorption to the membrane surface, and the change in retention with pH was related to charge characteristics (Nghiem *et al.*, 2005; Schäfer *et al.*, 2003). The charge of estradiol from pH 3 to 10 was predominantly neutral, though it became negatively charged above the dissociation constant ( $pK_a$  10.23) as it was a weak acid. Cellulose acetate was negatively charged at most pH values and it became increasingly negative at alkaline pH (Childress and Elimelech, 1996). The increase in negativity could be attributed to the dissociation of functional groups on the cellulose acetate surface. When estradiol was neutral adsorption could be attributed to hydrogen bonding between the hydroxyl functional groups, as they acted as both an electron donor and acceptor (Goss and Schwarzenbach, 2003). However, when estradiol was negatively charged at pH 11 and 12 electrostatic repulsion occurred between the filter and estradiol, and significantly reduced adsorption of estradiol on the filter. Consequently 80-100% of the total mass of estradiol was in the permeate.

The percentage of estradiol present in the permeate was dependent on filtrate volume. Using a range of volumes from 2 mL to 500 mL at pH 7 with an initial concentration of 100 ng/L the percentage of estradiol in the permeate increased as the filtered sample volume increased (Figure 2). At 2 mL the permeate only contained 20% of the total estradiol, while at 500 mL approximately 90% of total estradiol was present in the permeate, showing a 'breakthrough' phenomenon. However, significant losses of estradiol from the permeate are still observed at 50 and 100 mL sample volumes, with only 70-80% of total estradiol recovered in the permeate. To put these results in context, cellulose acetate syringe filters used for pre-treatment were often used to filter volumes of 10 mL or less (Furusawa, 1999; Karlsson *et al.*, 1999).

### Mass of Estradiol Loss

The mass of analyte lost to the filter was measured by desorbing analyte from the filter with methanol. Results were verified using mass balance. The mass of estradiol lost to 0.45 µm cellulose acetate filters were graphed as a function of pH and bulk organic matter type for 100 ng/L estradiol concentration (Figure 1 B and C) in comparison to total mass of analyte in the feed. Both the experimentally determined mass lost (based on methanol desorption) (Figure 1 B), and mass lost based on mass balance calculations (Figure 1 C) showed a general trend of approximately 50% estradiol retained until pH 10, which decreased greatly at pH 11 and 12 to between 0-20% estradiol retention.

Similar to the permeate results, the trend was related to the interaction of estradiol with the filter. However, with the exception of estradiol only and alginic acid experiments, the mass of estradiol desorbed from the filter using 10 mL methanol was lower than the calculated mass loss based on the mass balance. This difference was greatest for 5 mgC/L humic acid, as only 60–70% of adsorbed estradiol calculated from the mass balance could be desorbed by the methanol. For Australian NOM around 85% of estradiol was desorbed, while approximately 90-95% of estradiol was desorbed in the presence of 12.5 mgC/L humic acid and IHSS NOM. This suggested that in the presence of natural organic matter (with the exception of alginic acid) methanol was not efficient at desorbing the analyte.

Previous studies have demonstrated estradiol could interact strongly with bulk organic matter including NOM surrogates (Neale *et al.*, 2008; Yamamoto *et al.*, 2003). In addition, NOM surrogates, particularly humic acid, could adsorb to cellulose acetate membranes through hydrophobic interactions (Childress and Deshmukh, 1998). As a result the interaction of estradiol with bulk organic matter had an influence on the desorption of adsorbed estradiol from the filter using methanol. The organic matter-water partition coefficient ( $\log K_{OM}$ ) for estradiol and humic acid at pH 7 was 4.21 (Neale *et al.*, 2008), which was greater than the estradiol octanol-water partition coefficient ( $\log K_{OW}$ ), 4.01 (Hansch *et al.*, 1995; Nghiem *et al.*, 2004), indicating the preference of estradiol to sorb to humic acid compared to the solvent. Therefore, estradiol could remain bound to the filter in the presence of humic acid. In contrast,  $\log K_{OM}$  for alginic acid at pH 7 was 3.96 (Neale *et al.*, 2008), and as this was lower than  $\log K_{OW}$  it was likely any estradiol interacting with alginic acid would be desorbed by methanol, resulting in no difference between mass balance and experimental (methanol) desorption of estradiol from the filter in the presence of alginic acid.

## Conclusions

The results suggest that as much as 50% of the analyte estradiol in a sample could be lost to cellulose acetate 0.45 µm filters at pH 3 to 10. This was related to the adsorption of estradiol to the membrane in this pH range. Further, the presence of organic matter, particularly humic acid, reduced desorption of estradiol from the filter. Adsorption could be controlled by filtering the micropollutant when it was dissociated and hence charged. In the case of estradiol the pH could be adjusted to 11 prior to pre-filtration with cellulose acetate syringe filters. Alternatively, different filter material such as glass fibre filters (Holbrook *et al.*, 2004; Williams *et al.*, 2003) or hydrophilic HVLP (Rodriguez-Mozaz *et al.*, 2004) could be selected. However, further studies are required to assess the loss of steroidal hormones to different types of filter material as well as the impact of solute-solute interactions (Neale *et al.*, 2009). Results illustrated using the example of estradiol were applicable to other micropollutants, although the specific interaction with the cellulose acetate filter in the presence of organic and inorganic matrices need to be studied on an individual basis as such interactions are micropollutant specific. Previous studies have shown that other hormones, such as estrone, progesterone and testosterone behave similarly to estradiol (Nghiem *et al.*, 2004). The  $pK_a$  of estrone is 10.34 (Kwon *et al.*, 2006), therefore adsorption to the filter could be minimised above pH 10. However, progesterone and testosterone do not dissociate, and therefore pH adjustment could not be used to prevent the problem.

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## Author Disclosure Statement

The authors state that there are no competing financial interests exist.

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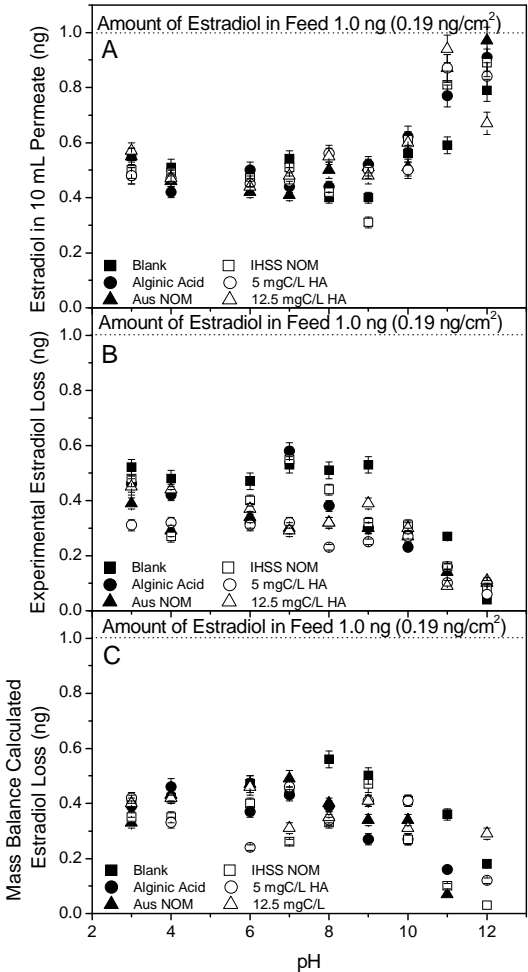
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List of Figures

Figure 1: A) Mass of estradiol in 10 mL of permeate, B) mass of estradiol lost to the filter determined by methanol desorption, and C) mass of estradiol lost to the filter calculated from the mass balance. The initial estradiol concentration was 100 ng/L and the organic matter concentration was 12.5 mgC/L, with the exception of humic acid which was also studied at 5 mgC/L (Background electrolyte of 1 mM NaHCO<sub>3</sub> 20 mM NaCl, HA is humic acid, and Aus NOM is Australian natural organic matter)

**Figure 2:** Percentage of estradiol present in the permeate as a function of solution volume (Initial estradiol concentration 100 ng/L, background electrolyte of 1 mM NaHCO<sub>3</sub> 20 mM NaCl, pH 7)

Figure 1



**Figure 2**

